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13. ABSTRACT *(Maximum 200 Words)*

The ability to detect carcinoma cells in the circulation of patients with breast cancer using a circulating cancer cell test developed by Cell Works and to characterize the isolated breast cancer cells using state-of-the-art multiple markers provides the ability to determine whether micrometastases have occurred and the progressive nature of the cancer. The test was designed to analyze single cells using immunofluorescence microscopy to produce molecular profiles that may be indicative of the metastatic potential of the breast circulating cancer cells. In particular, nuclear DNA can be quantified by measuring the fluorescence of bound DAPI, a DNA-specific dye, and compare the cancer cell DNA with reference WBCs on the same slide. Other markers can be assessed concurrently by quantitative immunofluorescence, such as Ki67, p27, p53, Her-2/neu, thymidylate synthetase, apoptosis, and hormone receptors (estrogen and progesterone), to further characterize the cancer cells. An ultimate goal is to commercialize a diagnostic and prognostic test to provide the patients and physicians with information that may be helpful with early metastatic cancer detection, elimination of lymph node dissection, early responsiveness of patients to hormonal therapy and/or systemic drug therapy.

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__ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

__ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Stephen Laska 6-30-00

PI - Signature Date

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5. INTRODUCTION

The focus of the research is to detect and characterize carcinoma cells in the circulation of patients with breast cancer using a circulating cancer cell test developed by Cell Works. It was critical to optimize the isolation, examination of markers, and imaging procedures of this test for breast cancer since the test was developed initially for prostate cancer. In particular, the scope of the research directly concerns the acquisition of key reagents and cells for the optimization of the methodology for aneuploidy measurements and staining of markers, such as growth/invasiveness, using breast cancer cell lines, whole blood samples, and ultimately, blood from breast cancer patients. Cell number and characterization information gathered from isolated breast circulating cancer cells in blood samples using this optimized test may be indicative of whether micrometastases are forming in the blood and, if present, the progressive nature of the cancer.

6. BODY

Significant progress has been made on the first two major objectives of this proposal, viz., to use cultured breast cancer cell lines to develop fluorescence markers for cell growth and invasive potential. Specific progress for each "TASK" in the statement of work is outlined below.

TASK 1. Acquisition of Reagents and Cell Lines.

A major change in our approach is that we now use total nuclear DNA content as a measure of aneuploidy rather than counting specific chromosomes detected with centromere probes. The fluorochrome 4, 6-diamidino-2-phrenylindole (DAPI) binds tightly to DNA with high specificity and the complex exhibits intense fluorescence. The basis for the quantitative assay is a comparison of the integrated fluorescence intensity, i.e., DNA content of a reference cell, WBC, with that of the cancer cell in question. Most circulating WBCs are in the G_0 phase of the cell cycle and have two copies of each chromosome (2n content of DNA). Normal epithelial cells would also have 2n DNA in G_0 or G_1 phase of the cell cycle and 4n DNA in the G_2/M phase. Therefore, a ratio of the cancer cell DNA content to the to the WBC DNA content of greater than 2.0 is a specific measure of aneuploidy. Ratios greater than 1.2 would also be indicative of aneuploidy of a non-dividing cancer cell, e.g., Ki67 negative cells. This assay is completely controlled internally since the nuclear DAPI fluorescence of the WBCs and the cancer cell are compared only on the same slide and measured within very close proximity on the slide. This eliminates any problems that may arise from staining, e.g., incubation time or DAPI concentration, or from image acquisition or image processing since the reference and test cells are always treated exactly alike.

Seven breast cancer cell (BCC) lines are now on hand and their reactivity with various fluorescence marker has been examined (see Task 2). The following monoclonal antibodies, conjugated to fluorescence labels, are now in hand: anti-cytokeratin-FITC, anti-cytokeratin-CY3, anti-Ki67-CY3, anti-P27-CY5, anti-KS-Texas Red, and anti-KS-FITC. Monoclonal antibodies for other markers, viz., anti-P53, anti- thymidylate synthetase, anti-Her2/neu, anti-Bcl-2, have been detected with fluorescently-labeled second antibodies. DNAase-CY5 has been used to detect and quantitate nuclear G-actin levels and dUTP-FITC has been used to label apoptotic breast cancer cells.

TASK 2. Optimization of Methodology for Aneuploidy Measurements and for Staining of Marker for Growth/ Invasiveness using BCC Lines

a. Measurement of Aneuploidy. Two cell lines, MCF7 and T47D were spiked into isolated WBCs, cytospun onto slides, fixed with 2% paraformaldehyde, stained with anti-cytokeratin-FITC and DAPI. Images were acquired with two different filter cubes that allow for discrimination of DAPI and FITC fluorescence. BCC were identified by positive cytokeratin staining. Experiments were done to show that DAPI fluorescence was linear with respect to exposure time and DAPI concentration. Image-Pro software allows for image processing and

measurement of the integrated fluorescence intensity of the DAPI-stained nuclei. Between 10 to 20 WBC nuclei in each of the images containing a BCC were used to determine the average integrated fluorescence intensity of the WBCs .The data for two breast cancer cell lines and a normal epithelial cell control are presented below in Table 1:

Table 1

	MCF7	T47D	CONTROL*
Number of cancer cells analyzed	55	45	30
Median cancer cell/WBC ratio	2.02	1.65	0.98
Mean cancer cell/WBC ratio	2.24	1.82	0.99
Range of cancer cell/WBC ratios	1.5-3.5	1.2-3.5	0.9-1.2

*Normal prostate cells kept 24 hrs without growth factors

The human karyotype is very tight, therefore aneuploidy is an excellent marker for identifying cells. Over 96% of the MCF7 cells had BCC/WBC ratios of 1.6 or greater and over 91% of the T47D cells had ratios of 1.3 or greater while all of the control cells had ratios of less than 1.2. Any circulating BCC whose BCC/WBC nuclear DAPI fluorescence ratio is greater than two (more than 4n content of DNA) should be considered neoplastic. The finding of any BCC in the peripheral blood should be suspect, especially if the cell has a BCC/WBC nuclear DAPI fluorescence ratio of 1.3 or greater. Such cells could be aneuploid if they are not in the growth phase of the cell cycle, e.g., negative staining for the nuclear antigen, Ki67. Rao et.al., (Cancer Epidemiology, Biomarkers & Prevention 7:1027-1033, 1998) did a quantitative study of multiple biomarkers in archival breast fine-needle aspiration specimens and reported that none of the benign cases were positive for G-actin and DNA content (5n) together and that none of the malignant cases were negative for G-actin and DNA together.

b. The ability to test the presence of hormone receptors in breast cancer is an important step to eventually correlate such information with clinical outcomes. In particular, studies were conducted to test for Estrogen Receptor (ER) and Progesterone Receptor (PR) in breast cancer.

   One particular study assayed six BCC lines for the presence of estrogen receptors and progesterone receptors. The results are summarized below in Table 2:

Table 2

BCC	TUMOR TYPE	TEST RESULTS	
		ER	PR
MCF7	Adenocarcinoma	+++	++/+++
T47D	Carcinoma, ductal	+	++++
Hs578t	Carcinoma	-	+
MDAMB468	Adenocarcinoma	-	+
MDAMB435	Carcinoma, ductal	-	+
ZR-75-1	Carcinoma, ductal	+	++++

c. Figure 1A shows a composite color image of a breast cancer cell that is positive for cytokeratin antibody, which is depicted by FITC or green staining of the cytoplasm; Figure 1B shows a positive breast cancer cell for progesterone staining. The progesterone antibody stains red in the nucleus due to the fluorophore Texas Red; Figure 1C shows both cytokeratin (green) and DAPI staining (blue nuclear staining); and Figure 1D shows a positive breast cancer cell for estrogen receptors. The estrogen receptor antibody is stained red using a goat anti-mouse Texas red conjugate.

d. Other Probe/Markers
The test is very versatile since many markers/probes can be tested on cancer cells. The test has the ability to test both experimental and state-of-the art technologies to gather additional information about breast cancer patients. Preliminary data could be generated using, for example, thymidylate synthetase, Her-2/neu, Bcl-2, and p53. One particular experiment illustrates the use of TUNEL staining for the detection of circulating cancer cell death for breast cancer cells. TUNEL staining measures nucleotide incorporation and provides a way to detect dying cells. Figure 2 shows color composite images of breast cancer cells, wherein Figure 2B shows positive staining for cytokeratin-CY3; and Figure 2A shows two cells staining positive for incorporated dUTP-FITC (green color) that is indicative of two dying cells.

TASK 3. Optimize test for cells in whole blood samples.

A variety of studies were conducted to optimize the isolation of cells in whole blood samples. These studies included testing optimal density gradients for isolating breast cancer cells, recovery experiments, storage and transportation studies, and validation studies for commercializing the test for breast cancer.

a. Table 3 (attached) is a summary of recovery experiments using two kinds of breast cancer cells, MCF-7 and T47D, that have been spiked into blood and isolated using a standard R&D isolation protocol. In Table 3A, 10 samples were tested for each cell line. The number of spiked cells was compared the number of cells found after the isolation procedure. Recovery rates ranged from 17.4% to 80.1% for a total of 20 samples with a median recovery rate of 58%. Table 3B shows a summary of the technologists assigned to the isolation, staining, and imaging steps. The recovery results were highly variable and the source or age of the blood samples could not be eliminated as variables. The problem of multiple cell layers found on some of the slide may have been associated with the source of the patients' blood, such as illnesses unknown to the company. Thus, no single team appeared to have more difficulty than the other teams. This study resulted in an evaluation of the isolation procedures for breast cancer cells, which led to the comparative study discussed below.

b. Table 4 (attached) depicts a comparative study of recovery rates between the optimal use of a second gradient, namely, 1.077 g/ml versus1.083 g/ml, using the MCF-7 breast cancer cell line. Both

groups used a first gradient of 1.068 g/ml and either 1.077 g/ml or 1.083 g/ml for the second gradient. Ten ml of blood was spiked with 50 MCF-7 cells. Ten samples were tested and the mean percentage recovery was 71.3% for the gradient of 1.077 g/ml (range of 61% to 85%) and 77.9% for the gradient of 1.083 g/ml (range of 69% to 86%). The recoveries for the second gradient of 1.083 g/ml showed equivalent or higher percentages of cells isolated versus the second gradient of 1.077 g/ml, which is normally used for the isolation of prostate cells in blood. Accordingly, the second gradient of the isolation protocol was revised from 1.077 g/ml to 1.083 g/ml. The BCC lines are smaller and denser than the prostate cancer cell lines so this modification of a different second density gradient was an important determination in order to optimize the isolation of breast cancer cells.

c. Table 5 (attached) depicts data for a storage and transportation study. The data show the detection of circulating breast cancer cells from blood of breast cancer patients. The same patient blood sample was aliquoted into two equal parts, which were designated as Group A and Group B. Group A samples were stored at room temperature for six hours and Group B samples were stored at room temperature for 24 hours. The same isolation and staining procedures were used for both sample groups. All isolated cells tested positive for cytokeratin antibody staining. After 24-hour storage and transportation, about a 16% loss of cell counts occurred with a loss of about 5% of positive cells.

d. Table 6 (3 pages, attached) is a summary of breast cancer validation studies conducted using the T47D and MCF-7 breast cell lines. In particular, Table 6 is a composite of 3 validation studies that provides statistical analysis of DNA aneuploidy measurements for 6 samples and 1 control per study or run. The DNA/WBC ratio determined per slide, the median, mean, and standard deviation of the DNA/WBC ratios are listed per sample. A DNA/WBC ratio greater than 1.2 for T47D cells and greater than 1.5 for MCF-7 is indicative of an aneuploid cell. In over 95% of the runs, the median DNA/WBC ratio was 1.7 or greater.

Figure 3 is a scatter plot of the recovery data from each sample performed in Exhibit 3-4A. The mean recovery rate of 71% was calculated for all samples. According to the validation protocol, a recovery greater than 50% would pass the validation. All sample recoveries were greater than 50%.

TASK 4. Work with blood from breast cancer patients. Correlate findings with clinical data.

a. Table 7 (attached) provides preliminary data showing the detection of circulating breast cancer cells from breast cancer patients' blood. Thirteen patients with Stage IV breast cancer were tested for the

presence of circulating cancer cells in a quantity of 20 ml of blood. The number of circulating breast cancer cells isolated ranged from 0 (2 patients) to 50 cells (1 patient). Eleven patient samples or 84.6% had circulating breast cancer cells.

b. A preliminary analysis of the Breast circulating cancer cell test from 20 Stage IV breast cancer patients was conducted to see whether a possible correlation between the number of cells isolated by the Cell Works Breast Circulating Cancer Cell Test and the patients' clinical status. Table 8 (6 pages, attached) shows a summary of the number of breast circulating cancer cells with clinical status. The CCCT was arbitrarily call a high test result if four or more cancer cells were isolated from the blood sample. If three or fewer cells were observed, the breast CCCT would be considered a low test result. A patient's clinical status would be considered "good" if her cancer was stable or improving. Conversely, the clinical status would be considered "bad" if the cancer were progressing. One patient was dropped from the analysis because her clinical status was designated as "stable disease?"

If the CCCT result was not correlated with clinical condition, then there is a high probability that the numbers in each of the boxes in Table 9A would be approximately equal and, consequently, the sum of the two numbers on each diagonal would be approximately equal. However, with the 19 patients discussed above, the sum along one diagonal is 13 (7+6) and along the other diagonal is 6 (2+4). Using Fisher's Exact Test with one tail, the odds of this occurring by chance alone was calculated to be 0.1149 or just over 11%.

Table 9A

CCCT	LOW (3 or less cells)	HIGH (> 4 cells)
STATUS		
Good	7 patients	4 patients
Bad	2 patients	6 patients

A "good" designation could be interpreted to mean only that the cancer is improving. A "bad" designation could be mean that the cancer were either progressing or stable. Thus, a new table below (Table 9B) suggests the following results. The sum along one diagonal is 15 (5+10) and the other is 4 (0+4). The correlation between the CCCT result and the clinical status now appears even stronger with a probability of less than .01 or 1%.

Table 9B

CCCT	LOW (3 or less cells)	HIGH (> 4 cells)
STATUS		
Good	5 patients	0 patient

Bad	4 patients	10 patients

Another analysis of the data with a further change to reduce the cutoff point by calling the CCCT result "low" if it was reduced to one cell or less. Thus, the new table (Table 9C) appears as follows:

Table 9C

CCCT	LOW (1 or 0 cells)	HIGH (>2 cells)
STATUS		
Good	5 patients	1 patient
Bad	3 patients	10 patients

Again, looking at the sums along the diagonals shows one is 15 and the other is 4. The new probability is calculated at .0237 or 2.4%. The correlation between the CCCT result and the clinical status has changed only slightly (1% versus 2.4%). Thus, the statistical test is said to be very strong against the change in definition. Exhibit 4-2 shows a summary of each cancer patients' age, sites of disease, current treatment, response, isolated Breast CCCT, and designations of "good" or "bad" and "high" or "low" as set forth above. At the present time, additional clinical information and follow-up testing are necessary to provide any relevance on these particular patients. Larger and longer studies are needed to correlate the number of circulating cancer cells and various probes with remission and progression of breast cancer.

7. KEY RESEARCH ACCOMPLISHMENTS

- Successful optimization of the breast circulating cancer cell test
- Successful validation studies of breast cancer using the circulating cancer cell test.

8. REPORTABLE OUTCOMES

- U.S. Patent Application Serial No. 09/430,175, filing date of October 29, 1999, entitled "Multiple Marker Characterization of Single Cells"

9. CONCLUSIONS

- Reagents and cell lines have been acquired and fluorescently-labeled antibodies have been prepared for detecting breast cancer cells and for examining growth, aneuploidy, and hormone receptor status.
- The test for circulating breast cancer cells has been optimized and validated with regard to detection, recovery and DNA aneuploidy using breast cancer cell lines spiked into 20 ml blood samples.
- The test has been used to isolate and detect cancer cells in 20 ml blood samples from breast cancer patients.

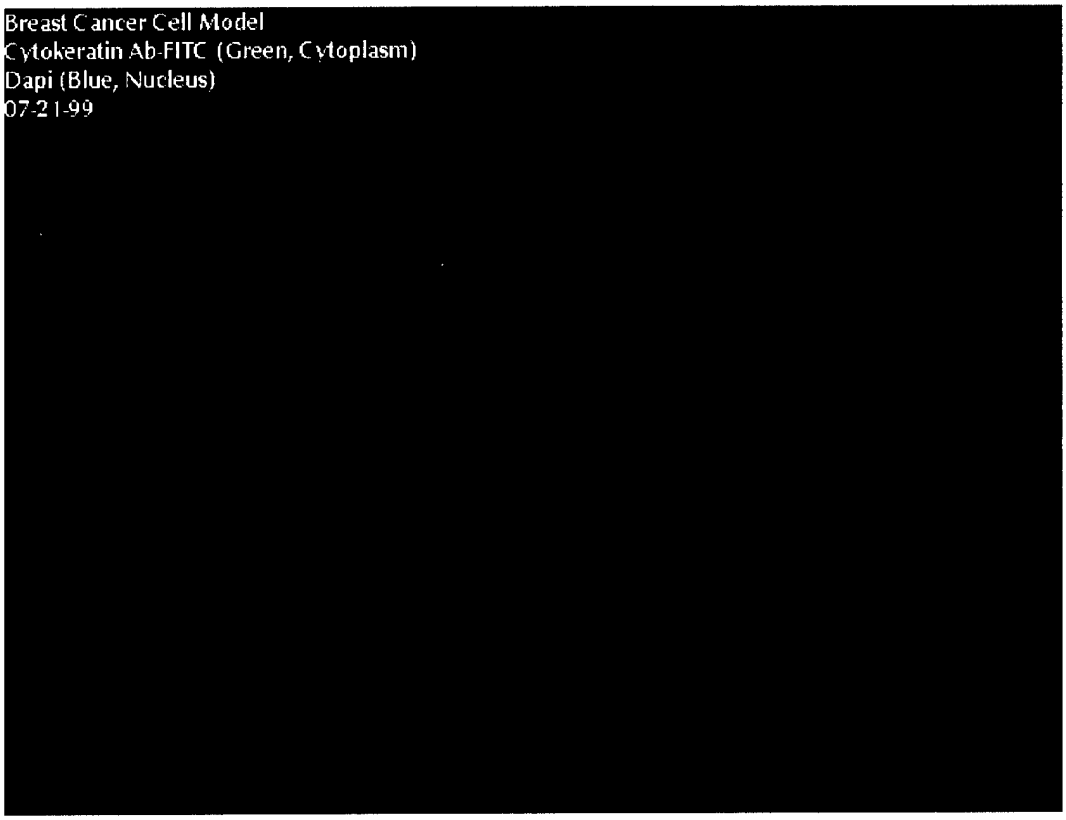
10. REFERENCES

None provided.

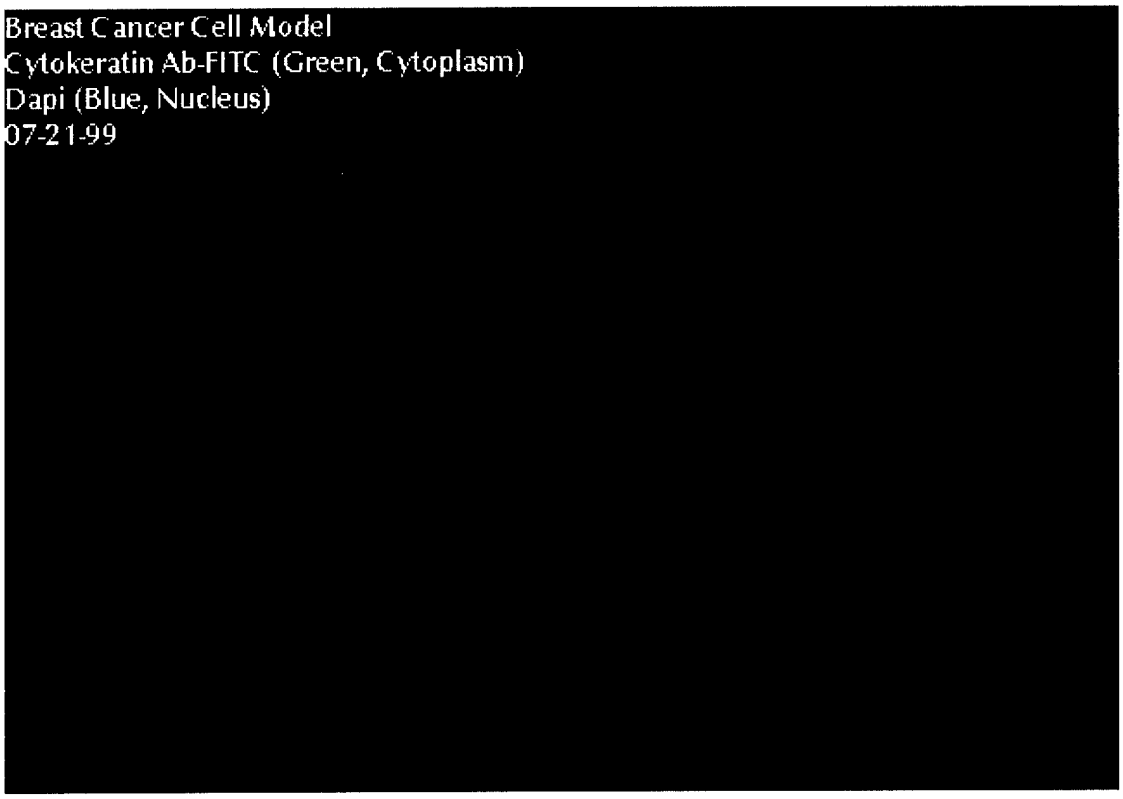
11. APPENDICES

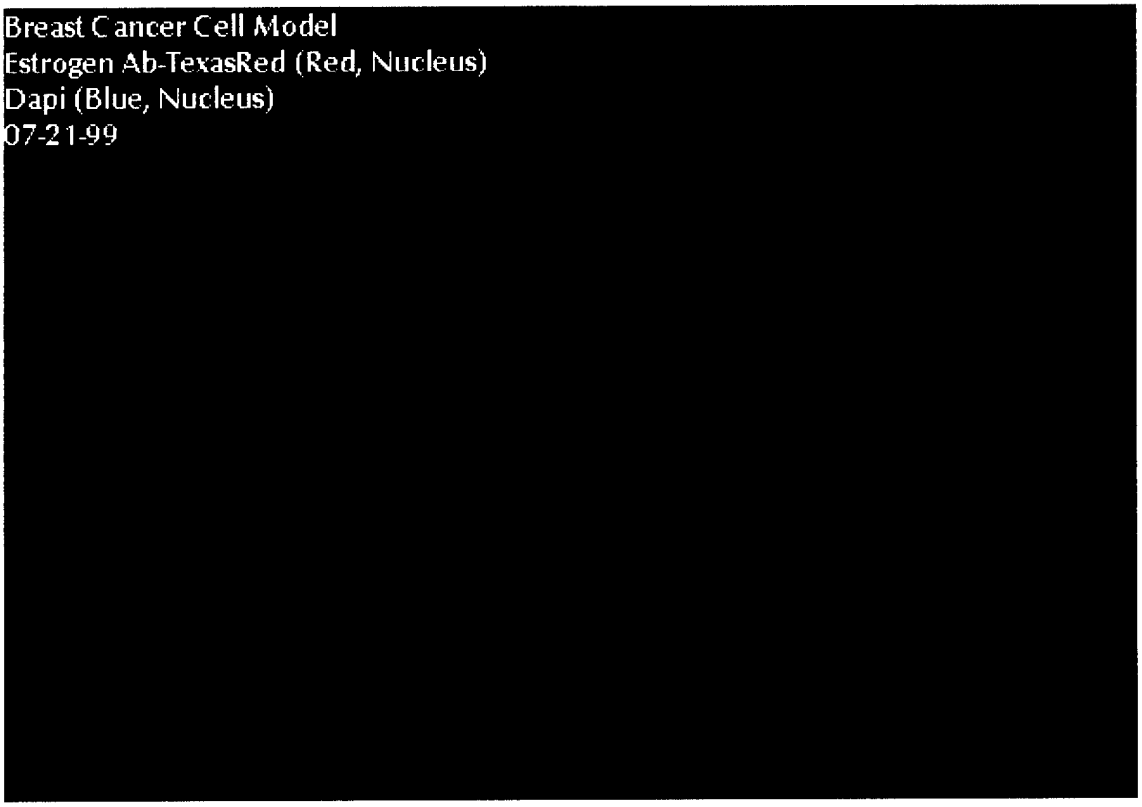
See attached pages 17 through 34.

Figures 1A & 1B



Figures 1C & 1D





FIGURES 2A & 2B

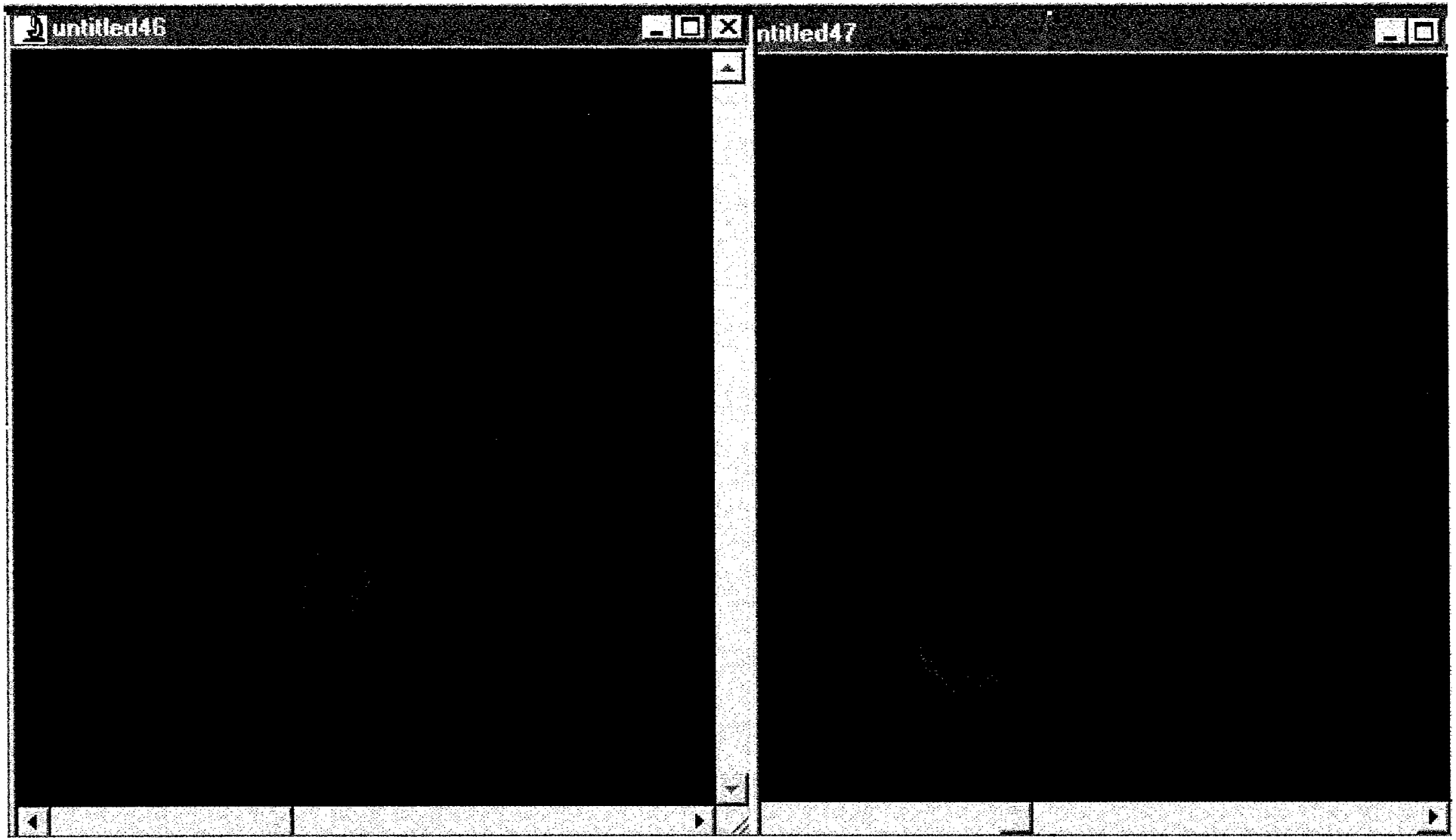
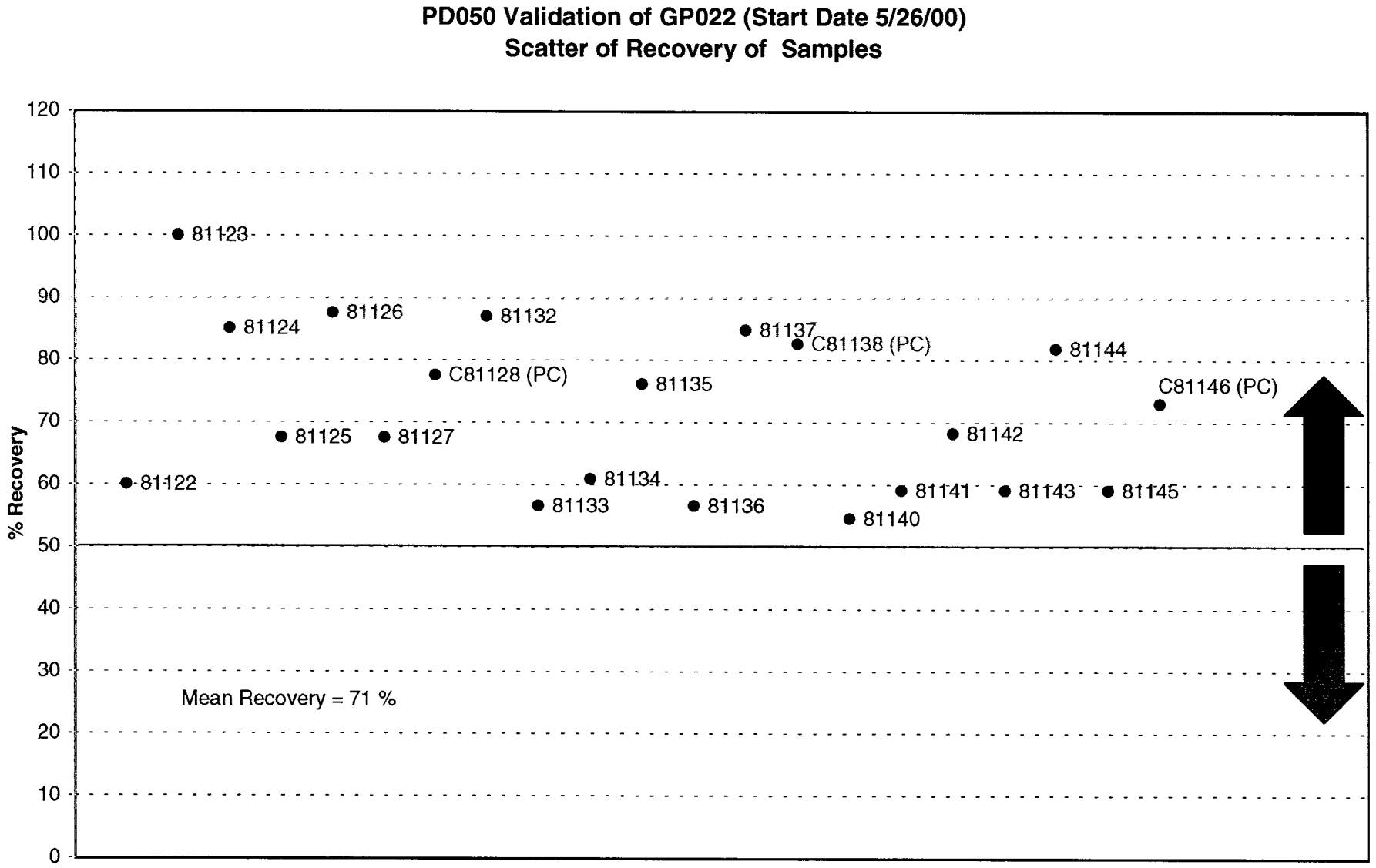


FIGURE 3


PD050 Validation of GP022 (Start Date 5/26/00)
Scatter of Recovery of Samples
% Recovery
120
110
100
90
80
70
60
50
40
30
20
10
0
81123
81124
81126
81132
81137
C81138 (PC)
81144
C81128 (PC)
81135
C81146 (PC)
81125
81127
81142
81122
81134
81141
81143
81145
81133
81136
81140
Mean Recovery = 71 %

Summary of Recovery Experiments on Breast Cancer Project

Based on R/D Protocol for Technology Transfer

TABLE 3A

- Breast cancer cell lines: MCF-7 and T47D
- Isolation SOP-PD033: The 2nd gradient of 1.077g/ml was replaced by 1.083g/ml gradient
- Staining SOP-PD034: Ab-WDZ-3-TxR was replaced by Ab-KS-TxR.
- Mixture of patient blood (in 5-ml EDTA tubes) from Johns Hopkins Hospital were used (2 tubes = 8-9 ml; 4 tubes = 18 ml)
- No of spiked cells were counted on spot (40 ul).

No.	Cell Lines	Blood Volume (ml)	Staining (=: Neg., +: Fair, ++: Good, +++: VG)			Comments	# Cells Spiked	# Cells Found	Recov. Rate%
			CK-FITC	KS-TxR	DNA-DAPI				
1	MCF-7	8	+++	+++	++		35	28	80.0
2		8	+++	+++	++		35	26	74.2
3		18	+++	++	++		35	24	68.6
4		18	+++	++	++		35	19	54.2
5		8	+++	=	++		155	105	67.7
6		8	++	+	++		155	107	69.0
7		8	+++	+	++	multiple cell layers on slide	155	40	28.4
8		8	+++	+	++		155	97	62.6
9		18	++	+	++	multiple cell layers on slide	155	72	46.5
10		18	++	+	++	multiple cell layers on slide	155	27	17.4
11	T47D	8	++	++	++		37	7	18.9
12		8	+++	++	++		37	29	78.3
13		18	+++	++	++		37	24	64.9
14		18	+++	=	++	multiple cell layers on slide	37	12	32.4
15		8	+++	+ / =	++		144	116	80.1
16		8	+++	=	++		144	110	76.4
17		8	+++	++	++		144	82	56.9
18		8	+++	+ / =	++		144	116	80.1
19		18	++	=	++	multiple cell layers on slide	144	44	30.6
20		18	+++	+	++		144	105	72.9
	40 ul of each spiked cell spot was stained with DAPI and counted		**Cell recovery rates ranging 17.4 – 80.1 % (N=20)**					**Ave. :**	**58.0%**

Summary of Recovery Experiments on Breast Cancer Project

Based on R/D Protocol for Technology Transfer

TABLE 3B

Patient Blood :	8ml	18ml	Cancer Cell spiked:	35-37	144-155
Recovery	64.3%	48.4%	Recovery:	58.9%	58.4%
	(N=12)	(N=8)		(N=8)	(N=12)

No.	Cell Lines	Blood Volume (ml)	Performer			Date of Isolation	Date. Staining	Comments	Recov. Rate%
			Isolation	Staining	Microscope				
1	MCF-7	8	Enzhi	Laura/Wilson	Laura	12/17/99	12/20/99		80.0
2		8	Cristine	Laura	Ping	12/17/99	12/20/99		74.2
3		18	Enzhi	Laura	Laura	12/17/99	12/20/99		68.6
4		18	Cristine	Laura	Ping	12/17/99	12/20/99		54.2
5		8	Enzhi	Laura	Cristine	12/15/99	12/16/99		67.7
6		8	Enzhi	Laura	Cristine	12/15/99	12/16/99		69.0
7		8	Cristine	Laura	CD/Enzhi	12/15/99	12/16/99	Multiple cell layers on slide	28.4
8		8	Cristine	Laura	Ping	12/15/99	12/16/99		62.6
9		18	Enzhi	Laura	Ping	12/15/99	12/16/99	Multiple cell layers on slide	46.5
10		18	Cristine	Laura	Laura/Ping	12/15/99	12/16/99	Multiple cell layers on slide	17.4
11	T47D	8	Enzhi	Laura/Wilson	Cristine	12/17/99	12/20/99		18.9
12		8	Cristine	Laura	Ping	12/17/99	12/20/99		78.3
13		18	Enzhi	Laura	Cristine	12/17/99	12/20/99		64.9
14		18	Cristine	Laura	Cristine/Ping	12/17/99	12/20/99	Multiple cell layers on slide	32.4
15		8	Enzhi	Laura	Laura	12/15/99	12/16/99		80.1
16		8	Enzhi	Laura	Ping	12/15/99	12/16/99		76.4
17		8	Cristine	Laura	Cristine	12/15/99	12/16/99		56.9
18		8	Cristine	Laura	CD/Ping	12/15/99	12/16/99		80.1
19		18	Enzhi	Laura	TG/Enzhi	12/15/99	12/16/99	Multiple cell layers on slide	30.6
20		18	Cristine	Laura	Laura	12/15/99	12/16/99		72.9
		40 ul cell spot	# Cells on spots were counted by Ping and Wilson at the end					Average recovery:	**58.0%**

TABLE 4

Comparative Study of Recovery Rates between Gradients of 1.077g/ml and 1.083g/ml Using MCF-7 Cell Line

Sample No.	1.077-Recovery	1.083-Recovery
#1	68%	70%
#2	75%	78%
#3	74%	79%
#4	85%	84%
#5	67%	78%
#6	65%	74%
#7	61%	69%
#8	81%	84%
#9	63%	86%
#10	74%	77%
N=10	71.3%	77.9%

1. 10 ml blood + 50 MCF-7 Cells;
2. Centrifugation at 1,340 rpm for 30 min.
3. CK staining for cell counting;

Table 5

Storage/Transportation Study:

Table A. Detection of Circulating Breast Cancer Cells from Breast Cancer Patients' Blood

CellWorks' No.	Patient No.	Quantity of Blood	Circulating Cancer Cells * A Group	B Group	Total Cell Counts
C967-C972	1343918	17 mL	1	0	1
C968-C973	2689536	16 mL	1	2	3
C969 A		20 mL	0	0	0
C970 G		20 mL	28	10	38
C971-C974	2856769	17 mL	176	174	350
(C979-C980	*1600166*	*16 mL*	*369*	*Failed***	*369)*
C977-C978	2856769	18 mL	33	32	65
C975-C976	0357585	17 mL	1	0	1
C981-C984	2582583	18 mL	2	1	3
C982-C986	0378845	18 mL	0	14	14
C983-C985	2854789	18 mL	4	0	4
C988-C999	2431427	18 mL	4	8	12
C989-C994	0202407	18 mL	20	7	27
C990-C995	0908020	18 mL	12 + 3clusters	8	20 + 3Clust.
C991-C997	2951951	18 mL	0	0	0
C992-C996	2851924	18 mL	1	7	8
C993-C998	3155514	14 mL	34	1	35
C1000-C1002	1988289	14 mL	0	0	0
C1001-C1003	2731957	18 mL	0	6	6
C1004-C1005	1692028	12 mL	0	0	0
C1006-C1007	3038711	18 mL	0	0	0
Total (N=21)			**320(689)**	**270**	**590(959)**
Loss Cells				**16%**	
Positive			**13(14) (65%)**	**12 (60%)**	**76%**

* The same patient blood sample was aliquoted into two equal parts which were classified into group A and B. Group A sample was stored at RT for six hours and Group B sample was stored/transported at RT for 24 hours. The samples in group A and B were processed by the same procedure.

** The blood was clouded after sample collection.

TABLE 6 (1 of 3)

PD050 (Start Date 5/26/00) DNA Ratios Statistical Analysis

Accession Number	Cell Type	DNA Ratios	Median	Mean	Standard Deviation
RUN 1 81122	T47D	1.4 1.9 2.0 2.1 2.2 2.3 2.3 3.3	2.2	2.19	0.54
81123	T47D	1.7 2.5 3.0 3.5 5.5	3.0	3.24	1.43
81124	T47D	1.2 1.7 1.8 2.5 3.1 3.4	2.2	2.06	0.74
81125	T47D	2.3 2.4 2.7 3.6 4.1	2.7	3.02	0.79
81126	T47D	1.6 1.8 2.7 3.0 4.9 5.6	2.9	3.27	1.64
81127	T47D	1.4 1.6 1.7 2.5 2.8 3.2	2.1	2.20	0.73
C81128	T47D	1.6 1.9 2.0 2.1 2.3 2.3 3.9	2.1	2.30	0.75

Table 6 (2 of 3)

PD050 (Start Date 5/26/00) DNA Ratios Statistical Analysis

Accession Number	Cell Type	DNA Ratios	Median	Mean	Standard Deviation
RUN 2 81132	MCF-7	1.7 1.7 2.1 2.6 2.7 3.3	2.4	2.35	0.63
81133	MCF-7	1.7 2.0 2.4 2.5 2.9 3.0	2.5	2.42	0.50
81134	MCF-7	2.4 2.5 2.6 2.6 3.3	2.6	2.68	0.36
81135	MCF-7	2.0 2.1 2.2 2.4 2.5	2.2	2.24	0.21
81136	MCF-7	1.6 1.7 1.9 2.1 2.1 2.1 4.2	2.1	2.24	0.89
81137	MCF-7	1.9 1.9 2.3 2.3 2.9	2.3	2.26	0.41
C81138	MCF-7	1.7 1.8 1.8 2.1 2.2	1.8	1.92	0.22

TABLE 6 (3 of 3)

PD050 (Start Date 5/26/00) DNA Ratios Statistical Analysis

Accession Number	Cell Type	DNA Ratios	Median	Mean	Standard Deviation
RUN 3 81140	T47D	1.3 1.3 1.7 1.8 2.3	1.7	1.68	0.41
81141	T47D	1.4 1.5 1.8 2.2 2.3	1.8	1.84	0.40
81142	T47D	1.4 1.7 3.3 3.8	2.5	2.55	1.18
81143	MCF-7	1.8 2.7 2.7 3.2	2.7	2.60	0.58
81144	MCF-7	1.5 1.9 2.2 3.0	2.1	2.15	0.64
81145	MCF-7	1.4 1.5 1.7 1.7 2.2 2.3	1.7	1.80	0.37
C81146	MCF-7	1.3 1.4 1.4 2.3 2.3	1.4	1.74	0.51

Median of all T47D Cells Analyzed for DNA Content	2.3
Mean of all T47D Cells Analyzed for DNA Content	2.4
SD of all T47D Cells Analyzed for DNA Content	1.00

Median of all MCF-7 Cells Analyzed for DNA Content	2.2
Mean of all MCF-7 Cells Analyzed for DNA Content	2.2
SD of all MCF-7 Cells Analyzed for DNA Content	0.56

Table 7

Detection of Circulating Breast Cancer Cells from Breast Cancer Patients' Blood

Date	Patient No.	Quantity of Blood	CirculatingCancerCells	Identification Markers
7/2/97	2838163	20 mL	10	KS + CK
7/2/97	2759901	20 mL	7	KS + CK
7/2/97	2979047	20 mL	50	KS + CK
7/9/97	2776257	20 mL	0	KS + CK
7/9/97	1292615	20 mL	4	KS + CK
7/9/97	2752135	20 mL	6	KS + CK
7/9/97	2932450	20 mL	2	KS + CK
7/9/97	2950069	20 mL	16	KS + CK
7/9/97	2592832	20 mL	27	KS + CK + CH.18
7/9/97	2992650	20 mL	3	EP4+ CK + CH.18
8/13/97	2992650	20 mL	0	EP4 + CK + CH.18
8/13/97	1963506	20 mL	5	EP4 + CK + CH.18
8/13/97	2392235	20 mL	4	EP4 + CK + CH.18

Table 8 (6 pages)

B = Bad = Progression
G = Good = Complete Remission, Partial Remission or stable disease

J9712: Sample Transport Study, Fall of 1999

History #	Date of Phlebot-omy	Age	Sites of Disease	Current Treatment	Response	Cell #	
3038711	10/8/99	36	NED (former L brachial plexus disease)	none	CR at 10/8	0	G
1692028	9/29/99	77	liver (heterogeneous enhancement)	biweekly Taxol 9/15, 9/29	SD 10/27	0	G
2731957	9/24/99	38	bone: spine, ribs, pelvis	Megace, Pamidronate	progressing 9/24 by bone scan, MRI, CA27.29	6	B
1988289	9/24/99	71	L lung, L pleura, bone	Arimidex, XRT to hip	stable 9/24	0	G
0202607	9/22/99	71	L infra and supraclav fossa, L pleura, bone, liver	biweekly Taxol 9/8, 9/22	progression in liver 10/6, brain mets	27	B
3155514	9/22/99	54	L axilla, B lung, liver mediastinum,	Taxoprexin 9/1	progression	35	B
2951951	9/22/99	54	L internal mamm node, adjacent sternum and rib	cycle 5 Cytox-Taxol 9/22	PR	0	G
2851924	9/22/99	48	bone, lung, liver, pelvic soft tissue, brain	weekly Taxotere 8/25, 9/1, 9/7 and brain XRT 8/99	meningeal spread, decreased hepatic lesions 11/99	8	B
0908020	9/22/99	44	R int mamm LN, mediastinum, pleural effusion, ribs, T and L spine, R SI joint	9/8 weekly Taxol and Arimidex	SD	20+3 clusters	G
2431427	9/22/99	62	pleural implants, L pleural effusion, sternum, L SI joint	cycle #3 Cytox-Taxol 9/22	9/22 decreased effusion, stable bone disease	12	G
0378845	9/17/99	75	bone: shoulder, C,T, and L spine, skull	Tamoxifen	SD	14	G

Table 8 (2 of 6)

B = Bad = Progression
G = Good = Complete Remission, Partial Remission or Stable Disease

2854789	9/17/99	37	bone: sternum, hip, and shoulder, lung, soft tissue, ocular mets	cycle #3 Zolodex and Pamidronate 9/17/99, XRT to eye one week earlier	SD?	4	Not Evaluable
2582583	9/17/99	42	pleura	Tamoxifen, Pamidronate	decrease in pleural effusion	3	G
1600166	9/10/99	73	multiple hepatic mets, RUQ ascites	Arimidex x 3 months	progression of hepatic mets	369+	B
2856769	8/31/99	69	extensive spine, pleura	weekly Taxol x 3, Pamidronate	SD	350, 65 on 9/7/99	G
0357585	9/1/99	67	suspicion L scapular met	no treatment	2x2 cm soft tissue mass next to sternum, rib destruction	1	G
2689536	8/31/99	77	ribs, L spine, manubrium	s/p cycle#1 Capecitabine	increasing pain, ? hospice	3	B
1343918	8/31/99	57	bone	Capecitabine, Pamidronate	decreasing CA 27.29	1	G
0516045	8/25/99	51	R inflamm breast cancer, 9x 13 cm	s.p neoadjuvant AC #1	response in breast	0	G
2851924	8/25/99	48	lung, multiple hepatic mets, pelvic soft tissue, bone	off treatment since 6/99	progression noted 8/14/99	38	B

Table 8 (3 of 6)

CCCT: L = low = 3 cells or less

H = High = 4 cells or more

J9712: Sample Transport Study, Fall of 1999

History #	Date of Phlebot-omy	Age	Sites of Disease	Current Treatment	Response	Cell #	
3038711	10/8/99	36	NED (former L brachial plexus disease)	none	CR at 10/8	0	L
1692028	9/29/99	77	liver (heterogeneous enhancement)	biweekly Taxol 9/15, 9/29	SD 10/27	0	L
2731957	9/24/99	38	bone: spine, ribs, pelvis	Megace, Pamidronate	progressing 9/24 by bone scan, MRI, CA27.29	6	H
1988289	9/24/99	71	L lung, L pleura, bone	Arimidex, XRT to hip	stable 9/24	0	L
0202607	9/22/99	71	L infra and supraclav fossa, L pleura, bone, liver	biweekly Taxol 9/8, 9/22	progression in liver 10/6, brain mets	27	H
3155514	9/22/99	54	L axilla, B lung, liver mediastinum,	Taxoprexin 9/1	progression	35	H
2951951	9/22/99	54	L internal mamm node, adjacent sternum and rib	cycle 5 Cytox-Taxol 9/22	PR	0	L
2851924	9/22/99	48	bone, lung, liver, pelvic soft tissue, brain	weekly Taxotere 8/25, 9/1, 9/7 and brain XRT 8/99	meningeal spread, decreased hepatic lesions 11/99	8	H
0908020	9/22/99	44	R int mamm LN, mediastinum, pleural effusion, ribs, T and L spine, R SI loint	9/8 weekly Taxol and Arimidex	SD	20+3 clusters	H
2431427	9/22/99	62	pleural implants, L pleural effusion, sternum, L SI joint	cycle #3 Cytox-Taxol 9/22	9/22 decreased effusion, stable bone disease	12	H
0378845	9/17/99	75	bone: shoulder, C,T, and L spine, skull	Tamoxifen	SD	14	H

SD = Stable Diease
PR = Partial Response

Table 8 (4 of 6)

2854789	9/17/99	37	bone: sternum, hip, and shoulder, lung, soft tissue, ocular mets	cycle #3 Zolodex and Pamidronate 9/17/99, XRT to eye one week earlier	SD?	4	?
2582583	9/17/99	42	pleura	Tamoxifen, Pamidronate	decrease in pleural effusion	3	L
1600166	9/10/99	73	multiple hepatic mets, RUQ ascites	Arimidex x 3 months	progression of hepatic mets	369+	H
2856769	8/31/99	69	extensive spine, pleura	weekly Taxol x 3, Pamidronate	SD	350, 65 on 9/7/99	H
0357585	9/1/99	67	suspicion L scapular met	no treatment	2x2 cm soft tissue mass next to sternum, rib destruction	1	L
2689536	8/31/99	77	ribs, L spine, manubrium	s/p cycle#1 Capecitabine	increasing pain, ? hospice	3	L
1343918	8/31/99	57	bone	Capecitabine, Pamidronate	decreasing CA 27.29	1	L
0516045	8/25/99	51	R inflamm breast cancer, 9x 13 cm	s.p neoadjuvant AC #1	response in breast	0	L
2851924	8/25/99	48	lung, multiple hepatic mets, pelvic soft tissue, bone	off treatment since 6/99	progression noted 8/14/99	38	H

± = Variable

Table 8 (5 of 6)

B = Bad, Stable Diease, Progression
G = Good, Remission, Partial Remission

J9712: Sample Transport Study, Fall of 1999

History #	Date of Phlebot-omy	Age	Sites of Disease	Current Treatment	Response	Cell #	
3038711	10/8/99	36	NED (former L brachial plexus disease)	none	CR at 10/8	0	G
1692028	9/29/99	77	liver (heterogeneous enhancement)	biweekly Taxol 9/15, 9/29	SD 10/27	0	B
2731957	9/24/99	38	bone: spine, ribs, pelvis	Megace, Pamidronate	progressing 9/24 by bone scan, MRI, CA27.29	6	G
1988289	9/24/99	71	L lung, L pleura, bone	Arimidex, XRT to hip	stable 9/24	0	B
0202607	9/22/99	71	L infra and supraclav fossa, L pleura, bone, liver	biweekly Taxol 9/8, 9/22	progression in liver 10/6, brain mets	27	B
3155514	9/22/99	54	L axilla, B lung, liver mediastinum,	Taxoprexin 9/1	progression	35	B
2951951	9/22/99	54	L internal mamm node, adjacent sternum and rib	cycle 5 Cytox-Taxol 9/22	PR	0	G
2851924	9/22/99	48	bone, lung, liver, pelvic soft tissue, brain	weekly Taxotere 8/25, 9/1, 9/7 and brain XRT 8/99	meningeal spread, decreased hepatic lesions 11/99	8	B
0908020	9/22/99	44	R int mamm LN, mediastinum, pleural effusion, ribs, T and L spine, R SI loint	9/8 weekly Taxol and Arimidex	SD	20+3 clusters	B
2431427	9/22/99	62	pleural implants, L pleural effusion, sternum, L SI joint	cycle #3 Cytox-Taxol 9/22	9/22 decreased effusion, stable bone disease	12	B
0378845	9/17/99	75	bone: shoulder, C,T, and L spine, skull	Tamoxifen	SD	14	B

SD = Stable Diease
PR = Partial Response

Table 8 (6 of 6)

Stable to Bad

2854789	9/17/99	37	bone: sternum, hip, and shoulder, lung, soft tissue, ocular mets	cycle #3 Zolodex and Pamidronate 9/17/99, XRT to eye one week earlier	SD?	4	?
2582583	9/17/99	42	pleura	Tamoxifen, Pamidronate	decrease in pleural effusion	3	G
1600166	9/10/99	73	multiple hepatic mets, RUQ ascites	Arimidex x 3 months	progression of hepatic mets	369+	B
2856769	8/31/99	69	extensive spine, pleura	weekly Taxol x 3, Pamidronate	SD	350, 65 on 9/7/99	B
0357585	9/1/99	67	suspicion L scapular met	no treatment	2x2 cm soft tissue mass next to sternum, rib destruction	1	G
2689536	8/31/99	77	ribs, L spine, manubrium	s/p cycle#1 Capecitabine	increasing pain, ? hospice	3	B
1343918	8/31/99	57	bone	Capecitabine, Pamidronate	decreasing CA 27.29	1	G
0516045	8/25/99	51	R inflamm breast cancer, 9x 13 cm	s.p neoadjuvant AC #1	response in breast	0	G
2851924	8/25/99	48	lung, multiple hepatic mets, pelvic soft tissue, bone	off treatment since 6/99	progression noted 8/14/99	38	B